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ORIGINAL ARTICLE

Proficiency of 38 HID-INDELS in kinship analysis and forensic parameters in a Mexican population

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Abstract
Introduction: Insertion–deletions for human identification (HID-INDELs) allow solving peculiar forensic situations when autosomal STRs are insufficient. Although limitations were predicted since the forensic implementation of biallelic markers, formal evaluation of these restrictions is scarce. Particularly, to define the informativity provided by HID-INDELs in kinship analysis is useful to avoid wasting work, resources, and –finally– disappointments.
Material and methods: For this reason, we analyzed the 38-plex HID-INDEL system in 25 Mexican families including father, daughter, and mother, whose kinship was previously established with 22 autosomal STRs.
Results and discussion: From genotypes of unrelated individuals, we updated allele frequencies and forensic parameters of the Jalisco state (West, Mexico), by increasing the population sample size from 62 to 112. Among the forensic *a priori* parameters, the Typical paternity index (PI) of the 38plex HID-INDEL system showed important differences regarding the PI and probability of paternity (W) estimated herein from real paternity cases, generally undervaluing the observed informativity of these 38-plex HID-INDEL system. Conversely, the studied HID-INDEL loci offered confident kinship conclusions based on the paternity index (PI $\geq$10,000) and probability of paternity (W $\geq$ 99.99%) in 68% of the standard trio cases (18/25), and only 12% of duo paternity cases (6/50) (motherless and fatherless). In fact, 14% of duo paternity cases (7/50) did not reach minimum requirements to stablish paternity (IP < 100; W < 99%).
Conclusions: We updated a Mexican population database for 38 HID-INDEL loci, and we described their proficiency from real paternity cases, detailing some limitations non-previously specified.

Abbreviations: INDEL, insertion-deletions; HID, Human Identification; STR, Short tandem repeats.

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PALABRAS CLAVE
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Eficiencia de 38 HID-INDELS en análisis de parentesco y parámetros forenses en una población mexicana

Resumen
Introducción: Las inserciones-deleciones para identificación humana (HID-INDEL), permiten resolver situaciones forenses peculiares cuando los STR autosómicos son insuficientes. Aunque se predijeron sus limitaciones desde la implementación forense de marcadores bialélicos, la evaluación formal de estas restricciones es escasa. Particularmente es útil definir su informatividad en el análisis de parentesco, para evitar desperdiciar trabajo, recursos y –finalmente– decepciones.
Material y Métodos: Por este motivo, analizamos el sistema de 38plex HID-INDELs en 25 familias mexicanas entre padre, hija y madre, cuyo parentesco se estableció previamente con 22 STR autosómicos. A partir de los individuos no emparentados, actualizamos las frecuencias alélicas y los parámetros forenses del estado de Jalisco (Oeste, México), aumentando el tamaño de la muestra poblacional de 62 a 112.
Resultados y discusión: Entre los parámetros forenses *a priori*, el índice de paternidad típico (IPT) del sistema 38plex HID-INDEL mostró diferencias importantes con respecto al IP y la probabilidad de paternidad (W) derivados de casos reales de paternidad, generalmente subestimando la informatividad observada de ese sistema. Sin embargo, los 38 HID-INDELs ofrecieron conclusiones de parentesco confiables basadas en el índice de paternidad (IP $\geq$ 10,000) y la probabilidad de paternidad (W $\geq$ 99,99%) sólo en el 68% de los casos tríos estándar (18/25), y el 12% de casos de paternidad dúo (6/50) (sin madre y sin padre). De hecho, el 14% de los casos de paternidad dúo (7/50) no alcanzó los requisitos mínimos para establecer la paternidad (IP <100; W < 99%).
Conclusiones: En este trabajo actualizamos una base de datos de población mexicana para 38 HID-INDELs y describimos su eficiencia en casos reales de paternidad, detallando algunas limitaciones no especificadas previamente.

Introduction

During the last decades, autosomal Short tandem repeat (STR) loci have constituted the markers of choice for Human identification (HID) purposes.[1] However, poor-quality samples commonly found in forensic casework generate partial STR profiles that complicate reaching conclusions (*e.g.* ancient or degraded DNA).[2] Similarly, the relatively high mutation rate of STRs generates sporadic inconsistencies in kinship analysis.[3] Although Single nucleotide polymorphisms (SNPs) have been implemented for HID purposes, they require instruments non-available in the majority of forensic genetic labs and/or involve no conventional methodologies.[2,4] Conversely, Insertion–Deletion length polymorphisms (INDELs) have been easily incorporated in forensic casework by means of short amplicon multiplexes followed by traditional capillary electrophoresis.[5–7] Particularly, both SNPs and INDELs biallelic loci have been useful for analysis of highly degraded skeletal remains,[8] and to deal with occasional STR mutations in paternity testing due to their low mutation rate.[9–11] Consequently, genetic systems based on HID-INDELs have been studied in worldwide populations to estimate allele frequencies and *a priori* forensic parameters, such as heterozygosity, power of discrimination (PD), power of exclusion (PE), and typical paternity index (IP), principally.[12–14] However, informativity weaknesses due to their biallelic condition have been described for kinship analysis, particularly in motherless duo-cases,[15] and when the alleged father is a close relative of the real one.[9,10,16] Interestingly, some parameters *a posteriori* such as the Paternity index (PI)[17] have demonstrated to be useful to evaluate the actual contribution of genetic markers in conventional kinship analyses,[18] which would be worthy to avoid wasting work, resources, and –finally– disappointments.

For this reason, we analyzed the 38-plex INDEL system in 25 Mexican families from the Jalisco state, including the father (F), mother (M), and daughter (D) with previously established kinship based on 22 autosomal STRs. The aims of this study were the following: 1) to evaluate the proficiency provided by the 38plex INDEL system to confirm kinship (*i.e.* PI) in these families fitted as standard trio and duo paternity cases (motherless and fatherless); 2) to update the forensic parameters of the studied Mexican population using INDEL genotypes from unrelated individuals and previously reported.[13]

Materials and methods

Studied sample

A total of 75 volunteers from 25 Mexican families from the Jalisco state (West, Mexico) were analyzed. Genetic relationships were previously confirmed with 22 autosomal STRs by the Powerplex Fusion kit (Powerplex Fusion System, Promega Corp.) displaying a PI $\geq$1.46E+12. These 25

families also constitute 25 standard paternity cases (trio), with 50 unrelated persons (fathers plus mothers), plus 25 individuals corresponding to the offspring. All individuals of each family signed a written informed consent agreeing to the ethical guidelines of the Helsinki Declaration. This study was approved by the local Ethical Research Committee of the Research Institute in Molecular Genetics.

Laboratory analysis

Genomic DNA was collected from bloodstain samples impregnated on FTA papers. Bloodstain punches of 1.2 mm washed with FTA Purification Reagent (Whatman™) and DNAsa-free water, were directly used as DNA samples for further analysis. Genotypes for the 38 autosomal INDELS were obtained using the PCR multiplex protocol previously described for this genetic system.[5] Separation and detection of PCR products were performed with an ABI Prism 3130 Genetic Analyzer, and using the GeneMapper v3.2 software (Applied Biosystems, Foster City, CA).

Data analysis

Firstly, for each Mexican family, the paternity index (PI) was estimated by INDEL locus and combined PI for each standard trio case (n = 25). Moreover, PI's were computed omitting one parent, adapting data as motherless and fatherless paternity duo-cases (n = 50). When the probability of paternity (W) was estimated, a flat *a priori* probability of 0.5 was taken into account for both paternity hypotheses: kinship and unrelated. With the INDEL genotypes of unrelated individuals, specifically fathers and mothers (n = 50), plus those previously reported (n = 62),[13] we were able to updated the forensic parameters of the Jalisco state (n = 112). The updated allele frequencies were used to compute the PI values with PATPCR, a free Excel spreadsheet designed by JA Luque (Barcelona, Spain, 2002). Although individuals of the paternity cases are included in the updated Mexican population database, a minimal effect is expected given the biallelic condition of INDEL markers. The Microsoft Excel spreadsheet Powerstats[19] was used to compute the following *a priori* forensic parameters: allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), power of discrimination (PD), typical paternity index (TPI), and polymorphic information content (PIC). However, the power of exclusion (PE) was computed with an specific formula for biallelic loci [PE = pq (1-pq)]. Finally, exact tests to assess Hardy–Weinberg expectations (HWE) and linkage disequilibrium (LD) between pairs of loci were performed using the GDA software.[20]

Results and discussions

Kinship informativity of the 38plex INDEL system

The complete 38-plex INDEL genotypes of the Mexican families are available in the Supplementary table S1. As expected by the low mutation rate of the INDEL loci,[9–11] any exclusion parent-daughter was observed, and PIs were estimated to evaluate the performance of the 38-plex INDEL system for kinship analysis (Supplementary table S2a and S2b). In duo paternity cases, the average PI values in fatherless (32,119) and motherless cases (40,716) were similar (P = 0.8579; t-student test), as could be expected. However, differences between PIs from trio and duo paternity cases were evident (P < 0.0001; t-student test), as shown in Fig. 1. Concerning the informativity by INDEL locus, R09 was the most informative locus in trio paternity cases with the largest range (average PI = 2.302; range = 0.5803–7.2254) (Table 1), which is associated with the extreme allele frequencies of R09 –closer to zero and one– that result in the largest and smallest PI values, respectively (Table 1). Based on duo paternity cases, R06 was the most informative INDEL locus (average PI = 1.5067). Conversely, although R03 displayed the minimum average PI (1.1407), R09 showed the smallest PI value (0.5803) (Table 1).

The generally accepted minimum standard PI for inclusion of paternity is $\geq$100,[21] but presently the majority of the labs attached to the English-Speaking Group of the International Society of Forensic Genetics (ESG-ISFG) require a PI$\geq$10,000,[22] which is probably required in most worldwide paternity testing labs. These PIs correspond to probabilities of paternity (W) of 99 and 99.99%, respectively, assuming a flat *a priori* probability (p = 0.5). From the studied Mexican families in standard paternity trio-cases, the 38-plex INDEL system displayed an average combined PI = 1.63E+05 (range: 1.09E+02 to 1.29E+06). However, 32% (8/25) of these PI values are below the limit required in most of the paternity laboratories (PI<10,000)[22] (Supplementary table S2a).

On the other hand, the Mexican families adapted as paternity duo-cases showed an average combined PI = 3.64E+04 (range: 5.98 to 9.55E+05), but only 12% of these values (6/50) reached the required limit to establish paternity (PI>10,000) (Supplementary table S2b). Even 14% (7/50) of these paternity duo-cases neither reached the minimum standard for inclusion of paternity (PI<100) [21] (Supplementary table S2b). In brief, the estimated performance of the 38-plex INDEL system to establish confident kinship from real standard trio and duo cases was only 68% and 12%, respectively.

These results highlight the role of the 38-plex system as a complementary genetic system to establish paternity. This is in agreement with the limitations described for biallelic markers to solve duo paternity cases,[15] and when the alleged father (AF) is a close relative of the real father.[9,10] For instance, to discard the four-step mutation hypothesis between the AF and child in the autosomal STR D22S1045, concerning the brother of the AF paternity hypothesis.[16] In this study, although the AF paternity hypothesis was favored with the 81 HID markers analyzed (LR = 13.4; W = 93.1%), 58 INDELs –by themselves– were not conclusive enough (LR = 110.3, W = 99.1%). Conversely, the 38-plex INDEL system has contributed successfully to solve complex paternity cases, such as supporting the hypothesis of maternal uniparental disomy of chromosome 2, despite the exclusions presence.[23]

The *a priori* forensic parameter for standard paternity cases, known as Typical paternity index (TPI),[24] was compared with its corresponding *a posteriori* analog based on the Mexican paternity cases studied herein (Fig. 1). By INDEL locus, the average TPI was minor than one (PI = 0.94; range: 0.6222 to 1.2727) (Table 1). This finding means that –theoretically– each INDEL marker commonly does not

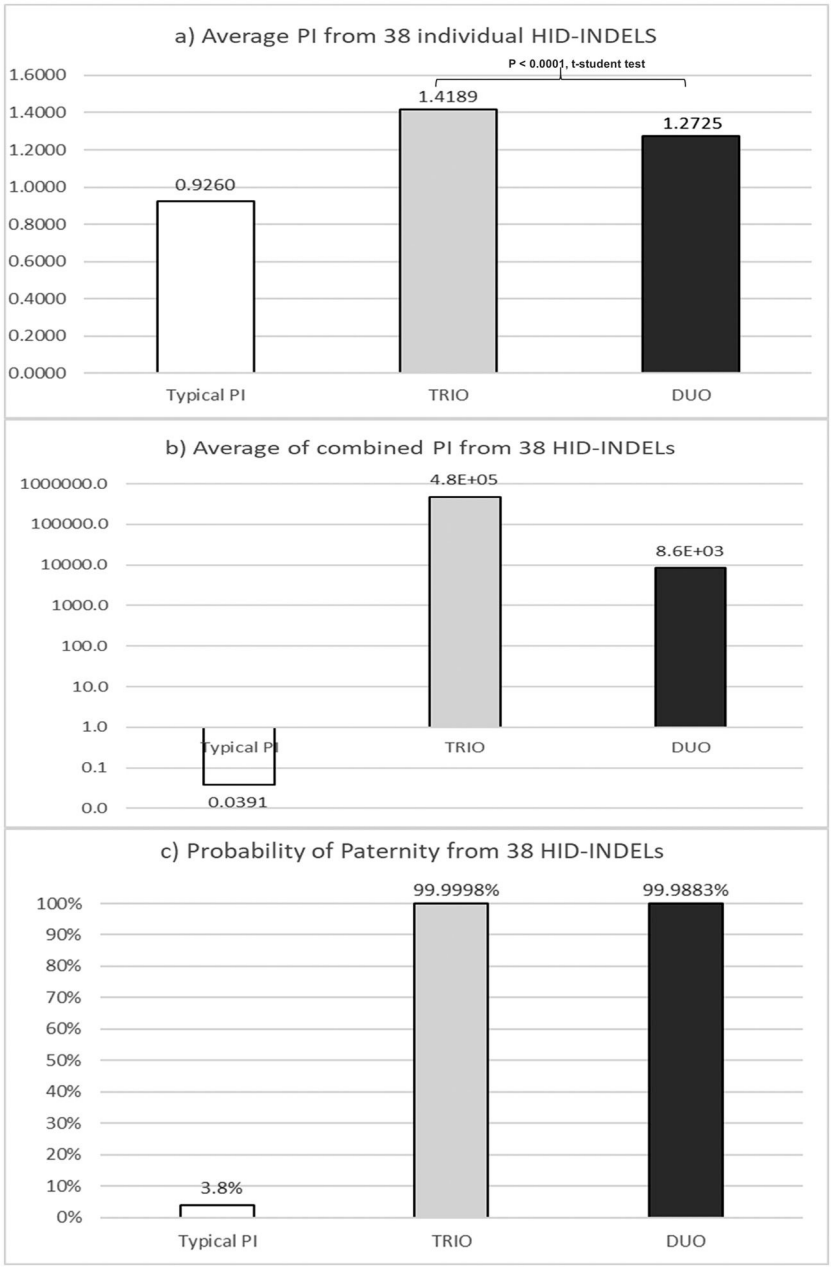

Fig. 1 Comparison of kinship results form typical paternity index (TPI) *versus* those from standard trio an duo paternity cases for the 38 HID-INDELs[5]: a) Average PI of individual markers; b) Combined from average PI of markes; c) Probability of paternity (W) from values described in incise b).

contribute statistically to confirm true paternities, despite the observed parent–child match (Fig. 1A). Conversely, the average PI estimated herein was larger than one, for both standard trio (PI = 1.4189; range = 1.2009–2.3055) and duo paternity cases (PI = 1.2725; range = 1.1407–1.5067) (Table 1). Consequently, INDELs contribute –modestly– to establish previously verified kinship (Fig. 1A). Interestingly, the product of the average TPI from the 38 INDELs did not support the paternity hypothesis (TPI = 0.0688; W = 6.43%) (Table 1; Fig. 1B, C). On the other hand, the product of the average PI from the 38 INDELs helped to verify the kinship in both trios (PI = 4.76E+05; W = 99.99979%) and duo paternity cases (PI = 8.58E+03; W = 99.98833%) (Fig. 1B, C). In brief, these results show that TPI undervalues the informativity provided by HID-INDELs –and consequently SNPs– to verify kinship; therefore, this *a priori* forensic parameter should be redesigned according to the low polymorphism of these biallelic markers.

Updating forensic parameters in a Mexican population

The INDEL genotypes from unrelated individuals studied herein (father and mother), were combined to the corresponding previously reported Mexican population dataset

Table 1 Comparison of the typical paternity index (TPI) and average PI from standard trio and duo paternity cases based on 25 families from a Mexican population.

INDEL		Typical[β]	TRIO (n = 25)	DUO (n = 50)[†]
Code[*]	**rs number**	**PI**	**Average PI by INDEL**	**Average PI by INDEL**
B01	**rs34541393**	0.9655	**1.5235**	**1.2463**
B02	**rs16624**	0.9180	**1.5175**	**1.2792**
B03	**rs2307689**	1.0370	**1.3022**	**1.2337**
B04	**rs35769550**	0.9655	**1.4727**	**1.2604**
B05	**rs2307700**	0.9032	**1.3363**	**1.2055**
B06	**rs140809**	0.8358	**1.4877**	**1.2938**
B07	**rs3047269**	0.9492	**1.4001**	**1.3059**
B08	**rs33972805**	0.9180	**1.6062**	**1.3519**
B09	**rs33917182**	0.8889	**1.2992**	**1.2012**
B10	**rs16402**	0.8750	**1.3040**	**1.2489**
G01	**rs1610871**	0.8750	**1.4759**	**1.2905**
G02	**rs2067238**	1.1915	**1.2822**	**1.2449**
G03	**rs2067294**	0.8750	**1.3398**	**1.1501**
G04	**rs2307710**	0.6829	**1.2209**	**1.2039**
G05	**rs2308242**	0.7887	**1.4236**	**1.4549**
G06	**rs2307580**	1.0566	**1.3932**	**1.2750**
G07	**rs1160956**	0.8889	**1.6314**	**1.4821**
G08	**rs34511541**	1.0370	**1.4065**	**1.2635**
G09	**rs2307978**	0.9655	**1.3680**	**1.3000**
Y01	**rs3051300**	0.9333	**1.3745**	**1.1478**
Y02	**rs10629077**	0.8116	**1.6599**	**1.1455**
Y03	**rs10688868**	1.0000	**1.2019**	**1.1573**
Y04	**rs2067208**	1.0769	**1.3113**	**1.2092**
Y05	**rs2307579**	0.8750	**1.4184**	**1.2132**
Y06	**rs2308020**	0.9180	**1.3237**	**1.2975**
Y07	**rs3080855**	1.0769	**1.2660**	**1.2667**
Y08	**rs1610919**	0.9180	**1.4220**	**1.3547**
Y09	**rs2307839**	0.7089	**1.6346**	**1.3791**
R01	**rs2308137**	0.9492	**1.3244**	**1.1913**
R02	**rs36040336**	0.9655	**1.2584**	**1.2187**
R03	**rs1160886**	0.9492	**1.2009**	**1.1407**
R04	**rs2308026**	0.8810	**1.5395**	**1.4064**
R05	**rs2307526**	0.9492	**1.2502**	**1.2943**
R06	**rs34811743**	0.8000	**1.5444**	**1.5067**
R07	**rs2308189**	0.9032	**1.4576**	**1.2952**
R08	**rs5895447**	1.0980	**1.5601**	**1.2445**
R09	**rs2308171**	0.6154	**2.0355**	**1.4297**
R10	**rs35605984**	1.1429	**1.3439**	**1.1613**
	Average	0.9260	**1.4189**	**1.2725**
Range	maximum	1.2727	2.3055	1.5067
	minimum	0.6222	1.2009	1.1407
	Combined PI[£]	**3.91E-02**	**4.76E+05**	**8.58E+03**
Paternity probability (W)[‡]		0.376	0.9999979	0.9998833

[*] The color code of the INDEL columns are related with the fluorocroms used during the PCR and detected in the capillary electrophoresis, and is in agreement with the original description.[5]

[β] Typical paternity Index (TPI) was added as reference for comparison purposes.

[†] Duo paternity cases include fatherless (n = 25) plus motherless (n = 25).

[£] Theoretical PI applying the product rule to the average PI of individual HID-INDEL loci.

[‡] Under a flat *a priori* probability of paternity ($p = 0.5$).

(Supplementary table S3).[13] Therefore, we updated the allele frequencies and forensic parameters of the 38 HID-Indels in the Jalisco state (Table 2), increasing the sample size from 62 to 112 individuals, improving the parameter's confidence. The average heterozygosities observed (Ho) and expected (He) in these 38 INDELs were 0.4505 and 0.4461, respectively. By INDEL, the observed heterozygosity (Ho) ranged from 0.1875 to 0.5804, whereas the expected heterozygosity (He) ranged from 0.2385 to 0.500, for the INDEL loci R09 and G02, respectively. Particularly, R09

Table 2 Allele frequencies and forensic parameters for 38 HID-INDELs in an updated Mexican sample from the Jalisco state (n = 112).

INDEL			Allele		Forensic Parameters						EHW
code	MID	rs number	Short	Large	He	Ho	PD	PE	IPT	PIC	p-value
B01	MID-2719	rs34541393	0.4732	0.5268	0.4986	0.4821	0.6320	0.1871	0.9655	0.3743	0.7819
B02	MID-185	rs16624	0.4063	0.5938	0.4824	0.4554	0.6268	0.1830	0.9180	0.3661	0.5225
B03	MID-1493	rs2307689	0.3929	0.6071	0.4770	0.5179	0.5926	0.1816	1.0370	0.3633	0.5403
B04	MID-2946	rs35769550	0.6071	0.3929	0.4770	0.4821	0.6105	0.1816	0.9655	0.3633	0.7406
B05	MID-1504	rs2307700	0.3393	0.6607	0.4483	0.4464	0.5958	0.1739	0.9032	0.3478	0.7706
B06	MID-520	rs140809	0.2902	0.7098	0.4119	0.4018	0.5716	0.1635	0.8358	0.3271	0.1563
B07	MID-2305	rs3047269	0.4955	0.5045	0.5000	0.4732	0.6373	0.1875	0.9492	0.3750	0.4806
B08	MID-3221	rs33972805	0.4063	0.5938	0.4824	0.4554	0.6268	0.1830	0.9180	0.3661	0.4150
B09	MID-2698	rs33917182	0.7009	0.2991	0.4193	0.4375	0.5697	0.1657	0.8889	0.3314	0.1578
B10	MID-116	rs16402	0.2857	0.7143	0.4082	0.4286	0.5612	0.1624	0.8750	0.3249	0.5238
G01	MID-785	rs1610871	0.3482	0.6518	0.4539	0.4286	0.6070	0.1754	0.8750	0.3509	0.5050
G02	MID-1151	rs2067238	0.5045	0.4955	0.5000	0.5804	0.5751	0.1875	1.1915	0.3750	0.4341
G03	MID-1209	rs2067294	0.3304	0.6696	0.4424	0.4286	0.5955	0.1723	0.8750	0.3446	0.3519
G04	MID-1514	rs2307710	0.2054	0.7946	0.3264	0.2679	0.4866	0.1366	0.6829	0.2731	0.6534
G05	MID-2050	rs2308242	0.2009	0.7991	0.3211	0.3661	0.4861	0.1348	0.7887	0.2695	0.3516
G06	MID-1384	rs2307580	0.5848	0.4152	0.4856	0.5268	0.5961	0.1839	1.0566	0.3677	0.2347
G07	MID-743	rs1160956	0.4866	0.5134	0.4996	0.4375	0.6500	0.1874	0.8889	0.3748	0.7175
G08	MID-3097	rs34511541	0.5446	0.4554	0.4960	0.5179	0.6116	0.1865	1.0370	0.3730	0.0475
G09	MID-1782	rs2307978	0.3571	0.6429	0.4592	0.4821	0.5926	0.1769	0.9655	0.3538	0.2453
Y01	MID-2648	rs3051300	0.3393	0.6607	0.4483	0.4643	0.5893	0.1739	0.9333	0.3478	0.7050
Y02	MID-2890	rs10629077	0.2545	0.7455	0.3794	0.3839	0.5423	0.1537	0.8116	0.3074	0.3713
Y03	MID-3277	rs10688868	0.3750	0.6250	0.4688	0.5000	0.5938	0.1794	1.0000	0.3589	0.9119
Y04	MID-1120	rs2067208	0.3661	0.6339	0.4641	0.5357	0.5694	0.1782	1.0769	0.3564	0.7053
Y05	MID-1383	rs2307579	0.2946	0.7054	0.4157	0.4286	0.5687	0.1646	0.8750	0.3293	0.4831
Y06	MID-1824	rs2308020	0.5759	0.4241	0.4885	0.4554	0.6328	0.1846	0.9180	0.3692	0.5888
Y07	MID-3114	rs3080855	0.3929	0.6071	0.4770	0.5357	0.5823	0.1816	1.0769	0.3633	0.0872
Y08	MID-834	rs1610919	0.6295	0.3705	0.4665	0.4554	0.6108	0.1788	0.9180	0.3577	0.9575
Y09	MID-1643	rs2307839	0.2366	0.7634	0.3612	0.2946	0.5257	0.1480	0.7089	0.2960	0.9675
R01	MID-1945	rs2308137	0.3080	0.6920	0.4263	0.4732	0.5636	0.1677	0.9492	0.3354	0.6950
R02	MID-2592	rs36040336	0.6607	0.3393	0.4483	0.4821	0.5818	0.1739	0.9655	0.3478	0.2713
R03	MID-649	rs1160886	0.3348	0.6652	0.4454	0.4732	0.5827	0.1731	0.9492	0.3462	0.4206
R04	MID-1830	rs2308026	0.4144	0.5856	0.4854	0.4324	0.6373	0.1838	0.8810	0.3676	0.4197
R05	MID-1330	rs2307526	0.3527	0.6473	0.4566	0.4732	0.5939	0.1762	0.9492	0.3524	0.9844
R06	MID-3220	rs34811743	0.7054	0.2946	0.4157	0.3750	0.5797	0.1646	0.8000	0.3293	0.3281
R07	MID-1997	rs2308189	0.5625	0.4375	0.4922	0.4464	0.6397	0.1855	0.9032	0.3711	0.9559
R08	MID-3031	rs5895447	0.4152	0.5848	0.4856	0.5446	0.5853	0.1839	1.0980	0.3677	0.7747
R09	MID-1979	rs2308171	0.1384	0.8616	0.2385	0.1875	0.3732	0.1050	0.6154	0.2100	0.3688
R10	MID-2806	rs35605984	0.4866	0.5134	0.4996	0.5625	0.5875	0.1874	1.1429	0.3748	0.8359

showed the lowest of PD, PE, TPI, and PIC values (37.3%, 10.5%, 0.6154 and 21%, respectively). Conversely, G07 showed the highest PD (65%), and G02 showed the highest PE, PIC, and TPI values (18.75%, 37.5%, and 1.19, respectively) (Table 2). Although the combined PD for the 38 INDELS in the Mexican population of Jalisco is large (99.9999999999997%), the combined PE is relatively low (99.93%) regarding what is provided by conventional autosomal STR kits in the same Mexican population.[25]

For all the 38 INDELs, the genotype distributions were in agreement with Hardy–Weinberg equilibrium (HWE) expectations in the Jalisco state population, excepting G08 (p = 0.0475) (Table 1). However, after applying Bonferroni's correction for multiple tests (0.05/38 = 0.0013), all INDEL markers agree with the HWE expectations. Similarly, LD exact tests discarded allelic association between all pairwise combinations for the 38 INDEL loci (data not shown). In brief, these results (HWE and LD tests) validate the confident use of the binomial expansion of (p + q)2 and the product rule, respectively, to calculate the random matching probability (RMP) of DNA profiles based on HID-INDEL in forensic casework and kinship analyses.

Conclusions

Based on the analysis of 38 HID-INDELs in 25 Mexican families, we evaluated the proficiency provided by the genetic system to verify paternity through the PI. Important limitations arose when one parent was omitted from the kinship test, emphasizing the complementary value of this HID system. The comparison of PI, *a posteriori* kinship

parameter, with the corresponding *a priori* parameter TPI, suggest that this should be redesigned for biallelic loci. Finally, we updated forensic parameters for the populations where the families come from (Jalisco state, Mexico).

Declaration of competing interest

Authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reml.2021.11.001.

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